Technische Universiteit
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Biomedical Engineering

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Protein Expression

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1 Protein Expression

Estimated bench time: 60 minutes
Estimated total time: 17 hours
Purpose: Protein expression of the bacteria.

It is essential to work near the Bunsen burner at all times.

1.1 Materials

- 1.5 mL cuvettes
- 2YT-medium
- Aluminum foil
- Antibiotic stock(s)
- Arabinose (20%)
- Cell Density Meter (OD600)
- Fresh culture of bacteria containing the right plasmid(s).
- Incubator
- IPTG (1 M)
- Pipettes and tips
- Sterile culture tubes

1.2 Protocol

- Prepare a 100 mL Erlenmeyer containing 25 mL LB and 25 µL of antibiotic stock.
- Pick bacterial colonies near the Bunsen flame with a pipette tip (another option is to continue with the picked bacteria which are verified by colony PCR).
- Load the colonies into correct labeled sterile culture tubes.
- Pipette up and down such that they are mixed well.
- Grow the bacteria in the incubator at 37 °C and 250 rpm overnight.
- Prepare a glycerol stock of the bacteria that contain the plasmids
    - Mix 300 µL of 50% glycerol with 700 µL of the bacterial culture, in cryo tube
    - Snap freeze the samples in liquid nitrogen and transfer them to the -80°C freezer
- Add 2ml kanamycin and the small culture to a 2L large culture in a 5L Erlenmeyer
- Grow the large culture in the incubator at 37 °C and 150 rpm.
- After 90 minutes: measure the OD600.
  OD measurement requires a blank measurement with 1 mL 2YT.
  Pipette 1 mL of the culture in the cuvette and measure the OD600.
- Put the culture back in the incubator (37 °C and 150 rpm). Regarding the fact that a cell division cycle takes around 20 minutes, calculate the amount of time the culture needs to obtain an OD600 of 0.6. (the OD600 doubles after ±20 minutes)
- After the additional time: measure the OD600 again. Pipette 1 mL of the culture in the cuvette and measure the OD600.
- When the OD600=0.6 add IPTG to culture tube.
- This makes the final concentration in the culture tube:
    - 1 uL of IPTG 1 mM per liter culture.
- Perform protein expression of ±15 hours at 18 °C and 250 rpm.
- Spin down the cells in 500 mL centrifuge bottles (fill bottles up to max. 80% of total volume) in the high-speed centrifuge, for 10 min at 10,000xg. Make sure all centrifuge

bottles are well weight-balanced, with a maximum weight difference of 0.05 g. Also make sure the rubber rings are present in the lids.

- Discard the supernatant
- Resuspend the pellets with Bugbuster (2 mL per 100 mL culture), benzonase (2 µL per 100 mL culture; 1:1000 compared to Bugbuster) and 1 mM TCEP, and incubate the cells for 1 hour at room temperature to extract the protein. Mixing can be performed on the shaking table
- Spin down the Bugbuster suspension in 35 mL tubes in the high-speed centrifuge, at 16,000xg for 20 min. Make sure all centrifuge bottles are well weight-balanced, with a maximum weight difference of 0.05 g. Also make sure the rubber rings are present in the lids. Follow the protocol for preparing the centrifuge as mentioned at the centrifuge
- Keep the supernatant as this contains the protein